SOME GENERAL COMMENTS ON CLONING AND THE USE OF THE P2 ROOM

Molecular cloning on the premises of the University is a privilege granted by the Biohazards Committee and revocable by them with any due cause. We expect every individual to comply completely with all restrictions and protocols; the laboratory supervisor is required to punish significant infractions by suspension of privileges.

You should familiarize yourself with all aspects of the P2 room, physical and biological containment, protocols for evaluating vector/host and procedures for preparing recombinant organisms. You will be given a simple practical exercise designed to demonstrate your ability to handle microorganisms satisfactorily; successful completion of this exercise will be required prior to use of the P2 facility and the reagents for molecular cloning.

Housekeeping within the P2 room is our responsibility. The room will not be cleaned regularly by the custodial staff. No debris should leave the room without prior autoclaving. If the material cannot be autoclaved, decontaminate it thoroughly by soaking in disinfectant (2% Wescodyne). Work surfaces, equipment and the floors should be cleaned with disinfectant whenever appropriate and whenever required by the guidelines.

Do not wear your regular labcoat into the P2 room. Exchange it at the door for a gown from the rack, and leave that gown on the rack when you leave. Not only is this good safety practice, it may reduce the accidental transmission of bacteria to other parts of our laboratory (eg., cubicles).

Water baths in the room should contain disinfectant and should be changed regularly.

Centrifuges and rotors should receive special care:

a. Inspect tubes and bottles for cracks before use.

b. Do not fill tubes to capacity; this increases the likelihood of spills.

c. Do not centrifuge tubes which are not capped in some manner.

d. Balance all tubes carefully.

e. Decontaminate rotors after use: fill with 70% alcohol, followed by disinfectant, followed by thorough rinsing with distilled water. Wash the centrifuge chamber with disinfectant after each use.

f. DO NOT AUTOCLAVE NITROCELLULOSE CENTRIFUGE TUBES: THEY EXPLODE. DECONTAMINATE WITH DISINFECTANT.

Bishop

P2 ROOM REGULATIONS

I. Laboratory Practices

A. Laboratory doors shall be kept closed while experiments are in progress.

B. Work surfaces shall be decontaminated daily, and immediately following spills of organisms containing recombinant DNA molecules.

C. All biological wastes shall be decontaminated before disposal. Other contaminated materials such as glassware, animal cages and laboratory equipment shall be decontaminated before washing, reuse or disposal.

D. Mechanical pipetting devices shall be used; pipetting by mouth is prohibited.

E. Eating, drinking, smoking and storage of food are not permitted in the laboratory area.

F. Persons shall wash their hands after handling organisms containing recombinant DNA molecules and when they leave the laboratory.

G. Care shall be exercised to minimize the creation of aerosols. For example, manipulations such as inserting a hot inoculating loop or needle into a culture, flaming an inoculation loop or needle so that it splatters and forceful ejection of fluids from pipettes or syringes shall be avoided.

H. Contaminated materials that are to be decontaminated at a site away from the laboratory shall be placed in a durable leak-proof container which is closed before removal from the laboratory.

I. Only persons who have been advised of the nature of the research being conducted shall enter the laboratory.

J. Children under 12 years of age shall not enter the laboratory.

K. The universal biohazard sign shall be posted on all laboratory access doors when experiments requiring P2 containment are in progress. Freezers and refrigerators used to store organisms containing recombinant DNA molecules shall also be posted with the universal biohazard sign.

L. An insect and rodent control program shall be instituted.

M. The use of laboratory gowns, coats or uniforms is required. Laboratory clothing shall not be worn to the lunch room or outside the building in which the laboratory is located.

N. Animals not related to the experiment shall not be permitted in the laboratory.

O. Use of the hypodermic needle and syringe shall be avoided when alternative methods are available.

P. The laboratory shall be kept neat, clean, and free of materials not pertinent to the research.

Q. Experiments of lesser biohazard potential can be carried out concurrently in carefully demarcated areas of the same laboratory.

II. Containment Equipment

Biological safety cabinents shall be used to contain aerosol-producing equipment such as blenders, lyophilizers, sonicators and centrifuges when used to process organisms containing recombinant DNA molecules, except where equipment design provides for containment of the potential aerosol. For example, a centrifuge may be operated in the open if a sealed head or safety centrifuge cups are used.

III. Special Laboratory Design

An autoclave for sterilization of wastes and contaminated materials shall be available in the same building in which organisms containing recombinant DNA molecules are used.

EMERGENCY PROCEDURES FOR THE P2 ROOM

A. ACCIDENTAL SPILLS

1. Restricted to work space. Pour a solution of 2% Wescodyne around the spill and allow it to flow into the area of the spill; paper towels soaked with disinfectant may be used to cover the area. Do not pour disinfectant directly on to the spill; demarcate it as described above.

2. Generalized spill - floor, etc., with aerosolization.

   a. Bar other individuals from entry.

   b. Remove protective garment and stow for autoclaving.

   c. Wash hands and face.

   d. Leave room and wait 30 min to allow dissipation of aerosols.

   e. Put on fresh gown and gloves; use of a surgical mask is also advisable.

   f. Demarcate the spill with 2% Wescodyne as described above. After area is completely soaked with disinfectant, allow 20 min contact time before clean up.

   g. Use autoclavable implements to clean up towels and other contaminated items; transfer these directly to autoclave; autoclave all implements used in clean-up.

B. FIRE

First attempt to extinguish the fire; a carbon-dioxide extinguisher is on the wall. If fire is out of hand, place contaminated materials into any handy container (centrifuge, covered pail, garbage can, autoclave, etc.) and split; call the fire department from outside the room.

C. INJURY

1. Small open injuries should be disinfected promptly.

2. If help is needed, use the telphone; then place contaminated material into covered container so that others can easily enter the room.

3. Accidental injection of contaminated material and all other accidents must be reported to Mike and to the University Biohazards Committee.